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Metabolizable energy content of wheat distillers' dried grains with solubles supplemented with or without a mixture of carbohydrases and protease for broilers and turkeys

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ABSTRACT

Two experiments were conducted to determine the apparent metabolizable energy (**AME**) and nitrogen-corrected AME (**AME$_n$**) of wheat distillers' dried grains with solubles (**wheat-DDGS**) without or with supplementation of an enzyme mixture containing xylanase, amylase and protease (**XAP**) in broilers and turkeys. One hundred twenty-six male Ross 308 broilers (Experiment 1) or 126 male BUT 10 turkeys (Experiment 2) were offered a nutrient-adequate diet from d 1 to 14. On d 14, birds in each experiment were allocated to six treatments consisting of three levels of wheat-DDGS (0, 300, or 600 g/kg) and two levels of XAP (0 or 250 mg/kg of diet) in a randomized complete block design. The AME or AME$_n$ content of wheat-DDGS was determined from the slope of regression of wheat-DDGS-associated energy intake (kcal) against wheat-DDGS intake (kg). In experiment 1, wheat-DDGS inclusion in the diets linearly decreased ($P < 0.05$) DM retention, AME and AME$_{n,}$ irrespective of XAP supplementation. The AME of wheat-DDGS without or with XAP for broilers was 3,587 or 3,700 kcal/kg DM, respectively and AME$_n$ was 3,356 and 3,459 kcal/kg DM for wheat-DDGS without and with XAP, respectively. In experiment 2, wheat-DDGS inclusion in the diet linearly decreased ($P < 0.05$) DM retention irrespective of XAP supplementation. Diet AME and AME$_n$ linearly decreased ($P < 0.05$) as the level of wheat-DDGS increased in the diets without added XAP, whereas there was no effect of increasing wheat-DDGS level on dietary AME or AME$_n$ in the XAP-supplemented diets. The AME of wheat-DDGS without and with supplemental XAP for turkeys were 3,355 and 3,558 kcal/kg DM, respectively and AME$_n$ was 3,109 and 3,294 kcal/kg DM, respectively, for wheat-DDGS without and with XAP. Supplemental XAP increased ($P > 0.05$) the AME and AME$_n$ of wheat-DDGS for broilers and turkeys by up to 6%. It was concluded that wheat-DDGS is a valuable source of AME for broilers and turkeys.

Keywords: broilers, DDGS, enzyme supplementation, metabolizable energy, turkeys

INTRODUCTION

The use of wheat for bioethanol production is expected to increase in the future (Batal and Dale, 2006). This will also increase the quantity of wheat Distillers Dried Grains with Solubles (**wheat-DDGS**) available as a feed ingredient for poultry. Wheat-DDGS is a viable feedstuff for poultry because nutrient (other than starch) levels are concentrated 3-fold in the wheat-DDGS after the starch fraction in the wheat is converted to ethanol (Nyachoti et al. 2005). Wheat is commonly used as a source of ME for poultry and it is likely that wheat-DDGS will also be a good source of ME for poultry. The apparent metabolizable energy (**AME**) and nitrogen-corrected AME (**AME_n**) contents of corn-DDGS have been determined in broilers (Batal and Dale, 2006; Adeola and Ilekeji, 2009) and the inclusion of corn-DDGS in diets for broilers and turkeys have been reported to support growth performance (Thacker and Widyaratne, 2007; Loar et al. 2010). Compared with corn-DDGS, there is insufficient information about the nutritive value of wheat-DDGS for broilers and turkeys. In addition, the use of wheat-DDGS for poultry may reduce competition between wheat demand for poultry and bioethanol production. In view of the possibility of using wheat-DDGS as a feed ingredient for broilers and turkeys, it is essential to determine its utilizable energy content.

Exogenous enzymes may ameliorate the anti-nutritive effects of non-starch polysaccharides (**NSP**) and phytate, and hence enhance the digestibility of feed ingredients and reduce nutrient excretion to the environment by poultry (Adeola and Cowieson, 2011; Woyengo and Nyachoti, 2011). The efficacy of exogenous enzymes to improve the nutritive value of bioethanol co-products has been determined mostly for bioethanol co-products derived from corn (Adeola and

Ileleji, 2009; Adeola et al. 2010). On the other hand, information about the value of exogenous enzymes to improve energy utilization in wheat-DDGS in broilers and turkeys is currently lacking in the literature. Development of nutrient matrix values for exogenous enzymes in wheat-DDGS will help in formulating diets that closely match bird requirement and prevent excessive surfeit.

The objective of the current study was to determine the AME and AME_n of wheat-DDGS without or with an enzyme mixture containing xylanase, amylase and protease (**XAP**) activities for broilers and turkeys.

MATERIALS AND METHODS

Animals and Management

The Scotland's Rural College's Animal Experimentation Committee approved all bird handling and sample collection procedures.

One hundred twenty-six male Ross 308 broilers chicks (Experiment 1) or 126 male BUT 10 turkeys (Experiment 2) were used for determination of AME and AME_n contents of wheat-DDGS. Birds had *ad libitum* access to the diets and water during the entire pre- and experimental periods and were reared in a house with facilities to control temperature, light, and humidity. In each of the experiments, the birds were offered a pre-experimental diet formulated to meet energy and nutrient requirements according to breeder recommendation for Ross 308 broilers (Aviagen, 2007) or BUT 10 turkeys (Aviagen, 2013), respectively. In each experiment, birds were allocated to experimental diets in a randomized complete block design using d 14 bodyweight as blocking criterion and transferred to metabolism cages on d 14. Each treatment had seven replicate cages and three birds per replicate cage.

Diets and Sample Collection

The pre-experimental diet offered from d 1 to 14 in experiment 1 and 2 contained (as-is), 3,035 kcal/kg of ME, 230 g/kg of CP and 6.8 g/kg of P. Six experimental diets were used in each of the two experiments. These diets consisted of a wheat-soyabean meal based reference diet containing no wheat-DDGS and two test diets containing 300 or 600 g/kg of wheat-DDGS, respectively and each of these three diets without or with added XAP (0.25 g/kg). At inclusion rate of 0.25 g/kg, the XAP supplied 2,000, 200 and 4,000 U/kg of xylanase, amylase and protease activities, respectively. The xylanase was a endo-1,4-beta-xylanase produced by a *Trichoderma longibrachiatum* and expressed in the same organism. The amylase was produced by *Bacillus amyloliquifaciens* and expressed in *Bacillus subtilis*. The subtilisin (protease) was derived from *Bacillus subtilis*. The three enzymes were produced separately and later blended to produce the xylanase-amylase-protease (XAP) admixture. One unit (U) of xylanase was defined as the quantity of the enzyme that liberates one mmol of xylose equivalent per minute. One unit of amylase was defined as the amount of the enzyme catalyzing the hydrolysis of one millimole glucosidic linkage per minute and one protease unit was defined as the quantity of the enzyme that solubilized one mg of azo-casein per minute. Energy-yielding ingredients such as wheat, soybean meal (SBM), gluten meal and soy oil were substituted with wheat-DDGS in a way that their ratios were the same across all the experimental diets.

Titanium dioxide (TiO_2) was added to the experimental diets (3 g/kg of diet) as an indigestible marker to enable determination of ME content by the index method. Experimental diets were offered from d 14 to 21 in both experiments. The ingredient and chemical compositions of the experimental diets used in both experiments are shown in Table 1. Excreta were collected daily from each cage for 3 d (d 18 to 20), dried and pooled for each cage prior to analysis.

Chemical analysis

Samples of diets, wheat-DDGS and excreta were analyzed for GE, DM, Ti, and N where necessary. Excreta were oven dried and ground to pass through a 0.5 mm screen using a mill grinder (Retsch ZM 100, F. Kurt Retsch GmbH & Co.KG, Haan, Germany) before chemical analysis. To determine DM content, samples were dried at 105°C for 24 hours (AOAC International 2006, method 934.01) in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England, UK). Gross energy was determined in a Parr adiabatic bomb calorimeter using benzoic acid as an internal standard (Model 6200, Parr Instruments, Moline, Illinois, USA). Nitrogen was determined by the combustion method (AOAC International 2006, method 968.06). Analysis for Ti was performed as described by Short et al. (1996). Xylanase activity in the experimental diets was measured using a kit (Megazyme International Ireland Ltd., Bray, Ireland) based on the method by McCleary (1991). Amylase activity was measured using Phadebas (Megazyme International Ireland Ltd.) tablets according to the method described by McCleary and Sheehan (1989). Protease activity was determined using the modified method of Lynn and Clevette-Radford (1984) with azo-casein as substrate.

Calculations and Statistical Analysis

Energy retention was calculated using the index method and AME was calculated as a product of energy retention coefficient and the gross energy content of the diet. Nitrogen-correction AME was calculated using the correction factor of 8.73 as the caloric correction factor for retained nitrogen (Titus, 1956).

Wheat-DDGS-associated AME intake was calculated following Adeola et al. (2010) procedures and described using the following equations: If the coefficients of AME for the assay diet, basal diet and test ingredient (wheat-DDGS) are represented by Cad, Cbd and Cti, respectively. Assuming additivity in diet formulation, the proportional contribution of energy by the basal (Pbd) and test ingredients (Pti) to the assay diet will be equal to 1. Mathematically; Pbd + Pti = 1 or Pbd = 1 – Pti.

Therefore;

1. $\text{Cad} = (\text{Cbd x Pbd}) + (\text{Cti x Pti})$

By solving for Cti,

2. $\text{Cti} = [\text{Cad} - (\text{Cbd x Pbd})]/\text{Pti}$

Substituting 1 – Pti for Pbd;

3. $\text{Cti} = \{\text{Cbd} + \left[\frac{\text{Cad}-\text{Cbd}}{\text{Pti}}\right]\}$

The product of Cti at each non-zero levels of wheat-DDGS substitution rates, the GE of wheat-DDGS and wheat-DDGS intake in kg is the wheat-DDGS-associated AME intake in kcal.

Data were analyzed using the Generalized Linear Models of Genstat Statistical Package (11th edition, VSN International). Statistical significance was set at $P < 0.05$ and tendency at $0.05 < P < 0.10$ for all mean comparisons. Dietary DM retention, AME and AME_n data were analyzed as a

3 × 2 factorial of wheat-DDGS inclusion level (0, 300 or 600 g/kg) and XAP (not added or added) using ANOVA procedures. Orthogonal contrasts were used to determine the differences in utilizable energy between the dietary treatments with different inclusion levels of wheat-DDGS and those without or with added XAP. The regression of wheat-DDGS associated AME or AME_n (kcal) against wheat-DDGS intake (kg) was done using regression analysis procedures. The slope of the linear regression equation represented the AME or AME_n value of wheat-DDGS. In the seven blocks, each consisting of six treatments (3 levels of wheat-DDGS and 2 levels of XAP), regression of wheat-DDGS-associated AME or AME_n against wheat-DDGS intake generated seven intercepts and seven slopes. The slope data were analyzed using ANOVA. The additional energy provided by the XAP supplementation was calculated as the differential between the slopes of diets not supplemented with XAP and those supplemented with XAP.

RESULTS

Dietary dry matter and energy retention

The chemical composition of the wheat-DDGS used in the current study is presented in Table 2. The analyzed CP, AA, crude fiber and gross energy contents in the wheat-DDGS were greater compared with wheat.

Dietary DM and energy retention for broilers receiving graded levels of wheat-DDGS without- or with added XAP in experiment 1 are presented in Table 3. For broilers, there were no wheat-DDGS × XAP interactions for diet DM retention, AME or AME_n. Increasing the inclusion level of wheat-DDGS from 0 to 600 g/kg in the diets linearly decreased ($P < 0.05$) DM retention and diet AME but decreased ($P < 0.05$) diet AME_n in a quadratic manner (Table 3). Supplemental XAP tended to improve ($P < 0.10$) DM retention, diet AME and AME_n.

Dietary DM and energy retention for turkeys receiving graded levels of wheat-DDGS without- or with added XAP in experiment 2 are presented in Table 4. For turkeys, there was no wheat-DDGS × XAP interaction for diet DM retention. Increasing the dietary inclusion level of wheat-DDGS from 0 to 600 g/kg linearly decreased ($P < 0.05$) DM retention, irrespective of XAP. There were wheat-DDGS × XAP interactions ($P < 0.05$) for diet AME and $AME_{n.}$ The interaction noted was because increasing the inclusion level of wheat-DDGS linearly decreased ($P < 0.05$) AME and AME_n in the diets not supplemented with XAP. On the other hand, AME or AME_n there was no effect of increasing wheat-DDGS inclusion level on diet in the XAP-supplemented diets.

Apparent metabolizable energy content in wheat distillers dried grains with solubles for broilers and turkeys

The AME and AME_n values of wheat-DDGS without- or with supplemental XAP for broilers are presented in Table 5. From the slope of the linear regression equations, the AME ± SEM (kcal/kg DM) of wheat-DDGS for broilers without- or with supplemental XAP were 3,587 ± 53 or 3,700 ± 81, respectively. Corresponding AME_n ± SEM (kcal/kg DM) were 3,356 ± 47 and 3,459 ± 71, respectively. Addition of XAP increased ($P > 0.05$) the AME or AME_n of wheat-DDGS for broilers by 113 or 103 kcal/kg DM, respectively.

The AME and AME_n values of wheat-DDGS without- or with supplemental XAP for turkeys are presented in Table 5. The AME ± SEM values (kcal/kg DM) of wheat-DDGS without- or with supplemental XAP for turkeys were 3,355 ± 108 or 3,558 ± 96, respectively. Corresponding AME_n ± SEM values (kcal/kg DM) were 3,109 ± 97 or 3,294 ± 85, respectively (Table 5). Supplemental XAP increased ($P > 0.05$) the AME or AME_n of wheat-DDGS for turkeys by 203 or 185 kcal/kg DM, respectively.

DISCUSSION

The objective of the current study was to determine the AME and AME_n contents of wheat-DDGS without or with added XAP for broilers and turkeys. Because the experiments were designed in a 3 × 2 factorial arrangement, this also afforded the determination of dietary DM retention, AME and $AME_{n.}$

The chemical characteristics of the wheat-DDGS used in the current study are comparable to those used in the study of Bolarinwa and Adeola (2012) as well as the mean values of 930 g/kg of DM, 380 g/kg of CP, 4,780 kcal/kg of GE, 77 g/kg of CF, 54 g/kg of EE, 344 g/kg of NDF, 139 g/kg of ADF and 53 g/kg of ash from eleven sources of wheat-DDGS (Olukosi and Adebiyi, 2013). Nonetheless, there is wide variability in the chemical composition of wheat-DDGS among sources (Olukosi and Adebiyi, 2013) which in turn may affect its nutritional characteristics for poultry.

Increasing the inclusion level of wheat-DDGS in the reference diet decreased DM retention, irrespective of XAP supplementation for broilers and turkeys in the current study. Bolarinwa and Adeola (2012) noted a linear reduction in DM and energy retention when 20% wheat-DDGS was incorporated in a wheat-SBM based diet for broilers. Similarly, Adeola et al. (2010) reported an average reduction in AME and AME_n of 23% with the inclusion of 600 g/kg of corn-DDGS in a corn-SBM reference diet for broilers. Adeola and Ileleji (2009) reported a 20% decrease in energy retention as the level of corn-DDGS increased to 60% in a corn-SBM reference diet for broilers. Dietary fiber reduces DM retention in broilers due to its low digestibility (Adeola et al. 2010). The increase in dietary fiber associated with increasing wheat-DDGS levels in the reference diet may explain the reductions in DM retention and energy retention noted in the current study.

Although, fiber hydrolyzing enzymes are used during bioethanol production to reduce mash viscosity, the concentration of NSP in corn-DDGS increases at least 3-fold compared with corn (Widyaratne and Zijlstra, 2007). The anti-nutritional effects of NSP for poultry are well described in the literature (Adeola and Bedford 2004; Choct et al., 2004). Carbohydrases are able to hydrolyze NSP into sugars that can be utilized by the bird (Bedford, 2000) whereas proteases help to improve protein utilization (Adeola and Cowieson, 2011). The wheat-DDGS used in the current study contained 389 g/kg of NDF that are substrates for carbohydrase enzymes. Xylanase and amylase or a combination of both enzymes have been shown to be effective in improving energy value and nutrient digestibility of wheat-based diets for poultry (Choct et al. 2004; Adeola and Cowieson, 2011).

Liu et al. (2011) reported a 20% reduction in hemicellulose levels and a 619 kcal/kg increase in AME in diets containing corn-DDGS when investigating the effect of supplemental xylanase on growth performance and nutrient digestibility in broilers. Also, addition of an NSP hydrolyzing enzyme to a diet containing 20% corn-DDGS significantly increased dietary AME for broilers in a study by Lee et al. (2010). Supplemental XAP tended to improve dietary energy retention in broilers and there was no effect of increasing wheat-DDGS inclusion level up to 60% in the diets supplemented with XAP for turkeys in the current study.

The AME value of wheat-DDGS for broilers was determined to be 3,587 kcal/kg DM in the current study. This value is greater than 2,653 or 2,216 kcal/kg DM for two wheat-DDGS samples, reported in the Bolarinwa and Adeola (2012) study, as well as the range of 2,144 to

2,868 kcal/kg DM for 10 samples of wheat-DDGS noted in Cozannet et al. (2010) study. It is common practice to correct the AME value of feed ingredients for nitrogen retention in order to account for variability in energy utilization that may occur due to differences in age and species of the animal as well as the protein quality of a diet. Correction for N retention resulted in a 6.4% reduction in the AME value of the wheat-DDGS for broilers in the current study which is similar to the 7% reduction reported by Bolarinwa and Adeola (2012). The AME_n value of wheat-DDGS for broilers was determined to be 3,356 kcal/kg DM in the current study. Similarly, the AME_n value determined in the current study was greater compared with the mean values of 2,278, 2,373 and 2,605 kcal/kg DM reported by Bolarinwa and Adeola (2012), Cozannet et al. (2010) and Vilarino et al. (2007), respectively for broilers. The AME and AME_n value of wheat-DDGS for turkeys was determined to be 3,355 and 3,558 kcal/kg DM, respectively in the current study. Cozannet et al. (2010) used the difference method in their study and determined the AME value of 10 samples of wheat-DDGS to range from 1,840 to 2,749 kcal/kg DM for turkeys. Furthermore, Cozannet et al. (2010) reported the AME_n values of wheat-DDGS for turkeys to range from 1,769 to 2,557 kcal/kg DM.

The gross energy in the wheat-DDGS used in the current study was greater compared with the average of those used in the study of Bolarinwa and Adeola (2012) (5,162 *vs* 4,517 kcal/kg DM, respectively). Nonetheless, energy metabolizability in the wheat-DDGS in the current study was 68% and was close to the 63% reported by Bolarinwa and Adeola (2012) for broilers. It appears therefore that the gross energy content of wheat-DDGS is influential in defining its AME value for broilers. On the other hand, although the gross energy content in the wheat-DDGS used in the current study were similar to those used in the study of Cozannet et al. (2010) (5,162 vs. 4,971 kcal/kg DM, respectively), energy metabolizability in the wheat-DDGS for turkeys was greater in the current study (65 vs. 47%, respectively). However, it is notable that the wheat-DDGS used in the current study contained greater levels of ether extract (7.4 vs. 4.7 g/kg) compared with those used in the study of Cozannet et al. (2010).

It was noted that the AME or AME_n values of wheat-DDGS were 232 or 247 kcal/kg DM, respectively, greater for broilers compared with turkeys in the current study. Similarly, Cozannet et al. (2010) observed that the mean AME and AME_n for 10 samples of wheat-DDGS were 127 and 208 kcal/kg DM, respectively, greater for broilers at 21 d of age compared with turkeys at 13 wks old. It is speculated that the difference in energy utilization in wheat-DDGS between broilers and turkeys in the current study is due to differences in physiological maturity between the two species at 21 d of age. However, this speculation is hardly supported by the similarity between the observations noted in the current study and the study of Cozannet et al. (2010) where the AME of wheat-DDGS for turkeys was determined at 13 wks of age. On the other hand, it is possible that the greater AME and AME_n for wheat-DDGS noted in the current study for turkeys compared with the study of Cozannet et al. (2010) are due to differences in the chemical characteristics of wheat-DDGS used.

The differences in reported energy values of wheat-DDGS show the need to develop a standardized method for determining energy value of wheat-DDGS for poultry. Although the differences in the nutrient composition of wheat-DDGS among sources might be implicated in causing variability to the utilizable energy value of the co-product, the methodology, age and species of poultry used for determining its energy value are also potential sources of variation.

Exogenous enzymes such as carbohydrases and proteases or a combination of these are often incorporated into poultry diets; however, there is a dearth of information on the efficacy of these enzymes to improve the nutritive value of wheat-DDGS. In addition to improving energy value and nutrient digestibility, supplementing diets containing wheat-DDGS with exogenous enzymes may reduce variability in the nutritive value of wheat-DDGS. The efficacy of exogenous enzymes to improve the nutritive value of bioethanol co-products has been determined mostly for corn-DDGS (Adeola and Ileleji, 2009; Adeola et al. 2010; Liu et al., 2011) but greater benefits may be derived from using exogenous enzymes in diets containing wheat-DDGS because wheat contains greater levels of NSP than corn.

In Adeola et al. (2010) study, a cocktail of xylanase and amylase increased the AME and AME_n of corn distillers grains by 5.7% and 6.2%, respectively. In the current study, the increases noted in the energy value of the wheat-DDGS due to XAP supplementation were marginal and were not statistically significant. The lack of significant XAP effect in the current study is least expected because feed ingredients or diets that contain substantial concentrations of fiber respond to a greater extent to carbohydrase supplementation (Bedford, 2000). Adeola and Cowieson (2011) noted a trend that indicated that the effects of carbohydrase supplementation are repressed when the energy value of the feed ingredient or diet being treated is high. The AME value of wheat-DDGS noted in the current study for broilers or turkeys were greater compared with other reported values in the literature (Cozannet et al. 2010; Bolarinwa and Adeola, 2012) and was also greater than the AME content of wheat. Perhaps, the high utilizable energy content in the wheat-DDGS used in the current study was partly responsible for the marginal effect of XAP. Also, analyzed xylanase and protease activities were approximately 20% lower than was expected in the XAP-supplemented diets for broilers and turkeys in the current study, and may be partly responsible for the marginal increment in AME in the wheat-DDGS noted. Nevertheless, considering that the wheat-DDGS contain substantial levels of soluble fiber, it is unlikely that a combination of carbohydrases and proteases will not significantly improve its utilizable energy for broilers and turkeys. It is therefore recommended that further studies be conducted to evaluate the efficacy of carbohydrases to improve the energy value of wheat-DDGS for broilers and turkeys.

In conclusion, the AME and AME_n (kcal/kg DM) contents of wheat-DDGS are 3,587 and 3,700, respectively for broilers whereas the AME and AME_n (kcal/kg DM) contents of wheat-DDGS for turkeys are 3,355 and 3,558, respectively. Supplemental XAP increased the metabolizable energy in wheat-DDGS by up to 203 kcal/kg DM for broilers or turkeys in the current study.

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Table 1. Analyzed nutrient composition of wheat distillers dried grains with solubles (as-is basis)

Item	g/kg
Dry matter	858
Crude protein	326
Gross energy (kcal/kg)	4,422
Crude fiber	80.0
Ether extract	72.5
Neutral detergent fiber	389
Acid detergent fiber	223
Ash	46.0
Calcium	1.10
Phosphorus	6.50
Potassium	11.3
Sodium	5.20
Amino acids	
Indispensable amino acids	
Arg	11.8
His	8.30
Ile	13.7
Leu	22.6
Lys	7.70
Phe	15.8
Thr	11.5
Met	4.50
Trp	3.80
Val	16.2
Dispensable amino acids	
Ala	14.0
Asp	18.3
Cys	5.90
Glu	84.9
Gly	14.9
Pro	30.2

Table 2. Ingredient and analyzed nutrient composition of experimental diets to determine apparent metabolizable energy content of wheat distillers dried grains with solubles without or with supplementation of a mixture of carbohydrases and protease for broilers and turkeys.

	Broilers			Turkeys		
Item	0	300	600	0	300	600
Wheat	561	385.2	209.2	484.5	328.9	173.5
Soybean meal (48% CP)	291.2	199.9	108.6	340	230.9	121.7
Soybean oil	54.2	37.2	20.2	30	20.4	10.7
Gluten meal[1]	31.6	15.7	0	58	32.3	6.6
Wheat-DDGS	0	300	600	0	300	600
Limestone (38% Ca)	18.5	18.5	18.5	13	13	13
Dicalcium phosphate[2]	14	14	14	35	35	35
Common salt	1	1	1	3	3	3
Vitamin/mineral premix[3]	3	3	3	4	4	4
DL-Methionine	1	1	1	1.5	1.5	1.5
L-Lysine HCl	2.5	2.5	2.5	6	6	6
Marker premix[4]	15	15	15	15	15	15
XAP premix	7	7	7	10	10	10
Total	1000	1000	1000	1000	1000	1000
Analyzed energy and nutrient composition[5]						
Dry matter, g/kg	880	880	870	883	883	874
Gross energy, kcal/kg	4,143	4,265	4,262	4,001	4,078	4,195
CP (N x 6.25), g/kg	226	256	276	258	277	293
Ca (calculated)	11.1	11.1	11.1	13.6	13.5	13.5
P (calculated)	6.2	7.2	7.9	10.6	11.2	11.9

[1]XAP premix replaced gluten meal at 7 g/kg.
[2]Contains 21.3% Ca and 18.7% P.
[3]Vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.2 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg.
[4]Contained 1 g of titanium dioxide added to 4 g of gluten meal.
[5]Values are means of duplicate analyses.

Table 3. Dry matter retention (%) and metabolisable energy (kcal/kg) for broilers receiving diets containing graded levels of wheat distillers dried grains with solubles without or with supplementation of a mixture of carbohydrases and protease[1].

	DM	AME	AME_n
DDGS effect			
0 g/kg of wheat-DDGS	72.7	3,609	3,394
300 g/kg of wheat-DDGS	65.1	3,322	3,131
600 g/kg of wheat-DDGS	60.9	3,250	3,059
S.E.	1.32	57.4	50.2
P values for DDGS effect	<0.001	<0.001	<0.001
XAP effect			
Without XAP	65.2	3,346	3,155
With XAP	67.2	3,442	3,227
S.E.	1.08	47.8	40.6
P values for XAP effect	0.062	0.063	0.057
DDGS × XAP interaction	0.920	0.976	0.982
P values for contrasts			
Diet (linear)	<0.001	<0.001	<0.001
Diet (quadratic)	0.142	0.059	0.038

[1]Average analyzed enzyme activities were 1421 U/kg of xylanase, 262 U/kg of amylase and 3064 U/kg of protease, respectively.

S.E - standard error of difference of mean

Table 4. Dry matter retention (%) and metabolisable energy (kcal/kg) for turkeys receiving diets containing graded levels of wheat distillers dried grains with solubles without or with supplementation of a mixture of carbohydrases and protease[1].

	DM	AME	AME_n
DDGS effect			
Without XAP			
0 g/kg of wheat-DDGS	67.3	3,293	3,090
300 g/kg of wheat-DDGS	63.7	3,175	2,946
600 g/kg of wheat-DDGS	54.3	2,872	2,658
With XAP			
0 g/kg of wheat-DDGS	64.4	3,132	2,922
300 g/kg of wheat-DDGS	62.9	3,126	2,902
600 g/kg of wheat-DDGS	56.7	3,019	2,801
Pooled S.E	1.39	61.3	57.6
P values for main effect and interaction			
P values for DDGS effect	<0.001	<0.001	<0.001
P values for XAP effect	0.699	0.681	0.622
DDGS × XAP interaction	0.170	0.038	0.015
P values for contrasts			
Without XAP			
Diet (linear)	<0.001	<0.001	<0.001
Diet (quadratic)	0.056	0.163	0.203
With XAP			
Diet (linear)	0.002	0.234	0.153
Diet (quadratic)	0.216	0.534	0.571

[1]Average analyzed enzyme activities were 1421 U/kg of xylanase, 262 U/kg of amylase and 3064 U/kg of protease, respectively.
S.E - standard error of difference of mean

Table 5. Regression equations for the apparent metabolizable energy content of wheat distillers dried grains with solubles without or with supplementation of a mixture of carbohydrases and protease for broilers and turkeys[1,2]

Measurements	Regression equation	SE of slope	r^2	P-value
Broilers				
AME, kcal/kg DM				
Without XAP	Y = 3,587X + 3.2	58.8	0.995	<0.001
With XAP[3]	Y = 3,700X – 2.5	87.5	0.989	<0.001
AME_n, kcal/kg DM				
Without XAP	Y = 3,356X + 4.9	52.3	0.995	<0.001
With XAP[3]	Y = 3,459X – 1.3	77.3	0.990	<0.001
Turkeys				
AME, kcal/kg DM				
Without XAP	Y = 3,355X + 48	91.3	0.985	<0.001
With XAP[3]	Y = 3,558X + 8.2	77.1	0.991	<0.001
AME_n, kcal/kg DM				
Without XAP	Y = 3,109X + 44	81.8	0.986	<0.001
With XAP[3]	Y = 3,294X + 9.6	68.1	0.992	<0.001

[1]AME and AME_n values of wheat-DDGS determined from regression of wheat-DDGS-associated AME or AME_n against wheat-DDGS intake; Y is in kcal, intercept is in kcal, and slope is in kcal/kg DM. The slope of the regression equation is the AME or AME_n value of the wheat-DDGS.
[2]Supplemental XAP did not significantly ($P > 0.05$) increase the AME or AME_n values of wheat-DDGS for broilers and turkeys
[3]Average analyzed enzyme activities were 1421 U/kg of xylanase, 262 U/kg of amylase and 3064 U/kg of protease, respectively
S.E - standard error of difference of mean